

Lichens as source of versatile bioactive compounds

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Abstract:

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Lichens represent unique symbiosis of fungi (mycobionts) and algae (photobionts). Living in extreme conditions they developed various compounds to survive. Many of these original compounds have proven biological activities (antibiotic, antimycotic, antiviral, antitumor, antioxidant, etc) . This paper is synthesis of currently known data about lichens extracts and their potential use in pharmaceutics and medicine.

Key words: lichens, bioactive compounds, phytomedicine

Introduction

Pharmaceutical industry is forced to continual chase and development of new pharmacological active molecules. Similar to higher plants, lichens are considered as potential source of novel biologically active compounds. Lichens are complex symbiotic associations between a fungus (mycobiont) and an alga (photobiont) with unique characteristics in plant kingdom. It is estimated that there are approximately 25,000 species of lichens (Chapman, 2009). They are proven as the earliest colonizers of terrestrial habitats on the earth with a worldwide distribution from arctic to tropical regions and from the plains to the highest mountains (Taylor et al., 1995). Specific, even extreme, conditions of their existence, slow growth and long duration (age of several thousand years) are result of numerous protective compounds against different physical and biological influences (Denton & Karlen, 1973). These metabolites are actors of lichen extracts' bioactivity of various importance for modern pharmaceutics and medicine. Short review of utilization of lichens back in time and modern

investigation of lichen extracts and components and their application are given below.

A brief history of lichen utilization

Throughout the ages, lichens are used in nutrition of many animals and humans during famine (for example, during Leningrad siege) (Richardson, 1988; Karagoz et al., 2009). The desert species *Lecanora esculenta* is considered as "biblical manna" (Trease & Evans, 1978). Since Egyptians, lichens were used as dyes, perfumes and remedies in folk medicine (Vartia, 1973). It is known that Romans dyed their togas with orchil, a purple pigment from *Rocella* sp. and crottal, brown pigment from *Parmelia*, *Ochrolechia* and *Evernia* sp. (Muggia et al., 2009). Lichens-dyed textile reached considerable economic importance in 18th century in some parts of the world as in the Canary Islands (Muggia et al., 2009). Litmus, a blue coloring matter from lichen fermentation, was used as dye for textile and beverages (Beecken et al., 1961). Also, paper strips impregnated with litmus, a water extracted

dyes from *Rocella* sp., are used as pH indicators in laboratories from ancient times till present. Extracts of some species of lichens, like *Evernia prunastri*, are contents of perfumes (Trease & Evans, 1978). Naturally, the most important and studied application of lichens is the one in traditional medicine for treatment of animals and humans. For instance, New Zealand Maori traditionally use long, pendulous species of *Usnea* for nappies and sanitary pads (Perry et al., 1999). Also, *Usnea* species have been used in Asia, Africa and Europe for pain relief and fever control (Okuyama et al., 1995). *Usnea densirostra*, known as “barba de la piedra” served as a cure for various disorders in Argentina’s folk medicine (Correche et al., 1998). Two lichen species, *Parmelia caperata* and *Umbilicaria* sp. are reported in study of Chilean traditional medicine (Munoz et al., 1981). *Ramalina thrausta* is used in Finland for treatment of wounds, athlete’s foot or other skin diseases and taken to relieve sore throat and toothache (Vartia, 1973). *Cetraria islandica* is ancient cough remedy known as “tonicum amarum” accepted as a mucilage drug (Muller, 2001). Various species worldwide are used in traditional medicine and said that they are able to cure dyspepsia, bleeding piles, diabetes, bronchitis, pulmonary tuberculosis, spermatorrhoea and other diseases of the blood and heart (Richardson, 1988).

Lichen metabolites

Generally, lichens metabolites can be divided into two groups: primary and secondary. Primary metabolites are proteins, lipids, carbohydrates and other organic compounds involved in lichen’s metabolism and structure. Secondary metabolites, also known as lichens substances, are produced mainly by the fungus and secreted onto the surface of the lichen’s hyphae either in amorphous forms or as crystals. They are in focus of this review.hhh

Lichen substances

Secondary metabolites are complex, but predominantly small molecules, which comprise up to 20% of lichen’s dry weight (Muggia et al., 2009). Structures of more than 1000 different lichen substances are determined to date and many of them are pharmaceutically relevant (Muggia et al., 2009). Secondary metabolites are products of polyketide pathway, mainly monocyclic and/or bicyclic phenols joined by an ester bond (depsides), both ester and ether bonds (depsidones) or furan heterocycle (dibenzofurans and usnic acid), antraquinones, xanthenes, chromones and secondary

aliphatic acids and esters (Stojanović et al., 2011). Some of them are produced by the fungus or the alga per se, while others are exclusively produced by synergistic action of both partners in lichens. Whole spectrum of lichen metabolites evolved for protective purposes against various physical and biological environmental factors (Denton & Karlen, 1973). Large amounts of phenolic compounds fungal melanins are synthesized and accumulated in the thallus in order to absorb UVB light and shelter the photobiont from excessive radiation (Gauslaa & Solhaug, 2001). These photoprotectors have great antioxidant capacity (Hidalgo et al., 1994, Fernandez et al., 1996) and can be used as preservatives in cosmetic products (Muller, 2001). Certain phenolic compounds protect lichens from herbivores (Lawrey, 1989). Their toxicity, feeding deterrence to insect larvae and nematocidal activity has been demonstrated (Ahad et al. 1991, Emmerich et al., 1993). Other lichen metabolites have antibiotic properties which prevent microbial degradation of the thallus (Emmerich et al., 1993). Some of lichen metabolites are involved in maintaining of the symbiotic equilibrium (Hunecck, 1999), while others dissolved rocks for better attachment of lichens (Seaward, 1997).

Biological activity of lichens

Lichen metabolites exert manifold biological activity. First, **antibiotic properties** of lichen extracts are known for decades. First study by Burkholder originated from 1944. Later, Vartia (1973) reported antimicrobial activity of several lichen species. According to wide screening of antimicrobial activity of lichen extracts, it seems that bacterial inhibitions can vary within the lichen extract, solvent used for extraction and bacteria tested. Rankovic et al. (2007a; 2007b) tested aqueous, acetone and methanol extracts of *Cladonia furcata*, *Parmelia caperata*, *Parmelia pertusa*, *Hypogimnia physodes*, *Umbilicaria polyphylla*, *Lasallia pustulata*, *Parmelia sulcata*, *Umbilicaria crustulosa* and *Umbilicaria cylindrica* from Serbia on six species of bacteria and ten species of fungi. The strongest activity was observed with methanol extracts of *Parmelia pertusa* and *Parmelia sulcata* and the weakest activity was manifested by *Parmelia caperata* and *Umbilicaria cylindrica*. Aqueous extracts of all tested lichen species were inactive. *Bacillus mycoides* was the most sensitive bacterial species tested, whereas *Candida albicans* was the most sensitive fungal species examined. Other studies monitored *Ramalina farinacea* and 69 species of lichens from New Zealand and showed

their inhibitory effect against a lot of bacteria such as *Bacillus*, *Pseudomonas*, *E.coli*, *Streptococcus*, *Staphylococcus*, *Enterococcus*, *Mycobacterium* (Esimone & Adikwn, 1999; Perry et al., 1999). Behera et al. (2005) reported that acetone, methanol and light petroleum extracts of lichen *Usnea ghattensis* were effective against *Bacillus licheniformis*, *B. megaterium*, *B. subtilis* and *S. aureus*. Also, Karagoz et al. (2009) evaluated aqueous and ethanol extracts of 11 different species from Turkey and determined potent antibacterial activity of aqueous extract of *Peltigera polydactyla* and ethanol extract of *Ramalina farinacea*. Chemical identification of anti-Gram-positive activities revealed evernic acid, vulpinic acid and hirtusneanoside as main actors (Lawrey, 1986; Rezanka & Sigler, 2007). Recently, Mitrović et al. (2011) studied antibacterial activity of methanol extracts of five lichen species (*Flavoparmelia caperata*, *Evernia prunastri*, *Hypogymnia physodes* and *Cladonia foliacea*). Two lichen species were tested for the first time (*Evernia prunastri* and *Cladonia foliacea*). The analysis of their antibacterial potential were performed on 15 strains of bacteria and revealed the strongest inhibitory effect, especially on Gram (+) bacteria, of *Hypogymnia physodes* and *Cladonia foliacea*.

Second, **antifungal activities** of certain lichen substances are also revealed. Manojlović et al. (2005) reported antifungal activity of the anthraquinone parietin isolated from *Caloplaca cerina*. Two years later, antifungal properties were observed in extracts of the Andean lichens *Protousnea poeppigii* and *Usnea rigida*, which contain divaricatinic acid, isodivaricatinic acid, usnic acid and 5-resorcinol (Schmeda-Hirschmann et al., 2008). Also, Mitrović et al. (2011) determined strong antifungal effect of *Evernia prunastri* and *Hypogymnia physodes*. While *Evernia prunastri* exerted the best effect on yeasts, *Hypogymnia physodes* were better on filamentous fungi.

Third, **antiviral properties** has been attributed to specific lichen secondary metabolites. For instance, Perry et al. (1999) showed antiviral activity of usnic acid against Herpes simplex type 1 and Polio type 1 viruses. Parietin extracted from *Teloschistes chrysophthalmus* proved as virucidal for Junin and Tacaribe arenaviruses (Fazio et al., 2007). Lichenan, widely distributed in lichens, demonstrated inhibition of tobacco mosaic virus (Stubler & Buchenauer, 1996).

Fourth, **antitumor activities** of lichens are of major importance as source of potential drugs for lethal malignant diseases. Usnic acid, the most extensively studied lichen metabolite since its first

isolation in 1844, exhibited an antiproliferative effect on human leukemia cells (K562) and endometrial carcinoma (HEC-50) cells (Carderelli et al., 1997, Ingolfssdottir, 2002, Krismundsdottir et al., 2002). Another lichen compound - depsidone pannarin inhibited cell growth and induces apoptosis in human prostate carcinoma DU-145 and human melanoma M14 cells (Russo et al. 2006, 2008). Lichen polysaccharide CFP-2 reduced the viability of HL-60 and K562 cells (Lin et al., 2003). Protolichesterinic acid isolated from *Cetraria islandica* showed inhibition of growth of breast cancer cell lines and mitogen-stimulated lymphocytes (Ogmundsdottir et al., 1998). Inhibition of enzyme 5-lipoxygenase involved in inflammation, non-specific binding to DNA polymerase β and DNA ligase I are possible mechanisms of antitumor activity of protolichesterinic acid (Muller, 2001). Bucar et al. (2004) revealed inhibitory activities on 12(S)-HETE inside antiproliferative effect of several lichen substances in human platelets.

Furthermore, **antipyretic and analgesic effects** of lichen components were demonstrated on animal studies. For instance, usnic acid from *Usnea diffracta* inhibited acetic-acid-induced writhing in mice and raised the pain threshold in dose-dependent manner (Okuyama et al., 1995).

Finally, **antioxidant properties**, already mentioned previously concerned with phenolic content of lichens. Jayaprakasha and Rao (2000) examined antioxidant capacities of methyl orsellinate, atranorin, osellinic acid and lecanoric acid. Bhattarai et al. (2008) noticed stronger antioxidant activities in lichens from Antarctic that the one in lichens from native to temperate or tropical regions. Mitrović et al. (2011) compared the chemical content of lichen extracts (*Flavoparmelia caperata*, *Evernia prunastri*, *Hypogymnia physodes* and *Cladonia foliacea*) and their free radical scavenging ability. They observed strong correlation according to previous conclusions of Ranković et al. (2010). *Hypogymnia physodes* with the highest phenolic content showed the strongest antioxidant effect.

Conclusions

Lichens represent powerful source of new bioactive molecules for various pharmaceutical purposes. Structure of more than 1000 lichen substances are available, but even more remain to be characterized, which is delayed by their natural occurrence in low concentrations. Lichens were frequently ignored by pharmaceutical industries because of their slow growth. Difficulties

encountered with collecting substantial amounts of plant materials may be circumvented by establishing lichen tissue-cultures that may be promising sources of novel biologically active compounds. Isolation into pure culture was attempted on 1,183 species of lichen-forming and lichenicolous fungi (Crittenden et al., 1995). However, it should be noted that lichen metabolites produced in mycobiontic cultures are not always identical to those produced from lichens themselves (Muller, 2001). As an alternative method, the transfer of genes responsible for the production of lichen metabolites are suggested (Huneck, 1999). Lichen tissue culture and gene transfer techniques may considerable enlarge the access to lichen derived substances and their excessive pharmaceutical screening. Eventual drug-promising molecules from lichens can be improved with increased stabilities and reduced toxicities by modern computer-aided drug design programs and combinatorial chemistry (Muller, 2001).

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